PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2005/001688	18.02.2005	20.03.2004

International Patent Classification (IPC) or both national classification and IPC
C12N15/52, C12N15/70, C12N9/88, C12P7/42, C12P13/02, C12R1/01

Applicant
DEGUSSA AG

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☒ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:

European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized Officer

BULCAO DE MELO BARRE

Telephone No. +49 89 2399-8972

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. II Priority

1. ☒ The validity of the priority claim has not been considered because the International Searching Authority does not have in its possession a copy of the earlier application whose priority has been claimed or, where required, a translation of that earlier application. This opinion has nevertheless been established on the assumption that the relevant date (Rules 43*bis*.1 and 64.1) is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-11
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-11
Industrial applicability (IA)	Yes:	Claims	1-11
	No:	Claims	

2. Citations and explanations

see separate sheet

Box No. VI Certain documents cited

1. Certain published documents (Rules 43*bis*.1 and 70.10)

and / or

2. Non-written disclosures (Rules 43*bis*.1 and 70.9)

see form 210

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

SECTION II

1. Priority (**Article 8 PCT**)

The present written opinion has been established with the assumption that the **priority date** 20.03.04 is validly claimed. Therefore, document WO 04/067738 (12.08.04), has not been considered to be part of the prior art as defined in the regulations (**Rule 64 (1) and (3) PCT**).

SECTION V

2. Cited Documents

D1: Journal of Biochemistry, Vol 125, No. 4, 1999, pages 696-704
D2: WO 95/12662
D3: Analytical Biochemistry, Vol 300, No. 2, 2002, pages 185-191

3. Novelty (**Article 33(2) PCT**)

The present application discloses the expression of a nitrile hydratase (NHase) in a two-vector expression system, wherein each of the NHase subunits alpha and beta is expressed separately, but simultaneously, by a different plasmid.

The subject-matter of the present application does not appear to be disclosed in the prior art as defined in the regulations (**Rule 64 (1)-(3) PCT**).
Therefore, in view of such prior art the subject-matter of the present application (**claims 1-11**) has to be regarded as being new (**Article 33(2) PCT**).

4. Inventive Step (**Article 33(3) PCT**)

The present application does not satisfy the criterion set forth in **Article 33 (3) PCT** because the subject-matter of **claims 1-11** does not involve an inventive step (**Rule 65 (1) and (2) PCT**).

The **closest prior art** to evaluate the inventiveness of **claims 1-11** is document **D1**. D1 discloses a one-vector system for expressing nitrile hydratases from *Rhodococcus* sp in *E. coli*, wherein the amplified NHase alpha and beta subunit genes are cloned into pUC18/19 and pET vectors under the control of the lac promoter. *E.coli* JM109 transformed with said vectors is cultured to produce the dimeric NHase.

Starting from **D1**, the underlying **technical problem** to be solved by the present application can be considered to lie in the provision of an alternative expression system for expressing the dimeric enzyme nitrile hydratase.

The **solution** provided by the Applicant to solve the above problem is a two-vector expression system wherein each of the NHase subunits alpha and beta is expressed separately, but simultaneously, by a different plasmid.

Document **D2** discloses a two-vector expression system for heterologously expressing recombinant dimeric enzymes in *E. coli.*, comprising constructing a first vector containing DNA encoding one of the subunits of the dimeric enzyme and then constructing a second vector containing DNA encoding the second subunit of the enzyme. Transformation of a prokaryotic host with said vectors, and culture of the transformed host produces the dimeric enzyme.
Although the two-vector system of D2 is exemplified with human CKMB creatine kinase, it is stated that said two-vector system is suitable for any recombinant enzyme consisting of two subunits.

Document **D3** also discloses a two-plasmid expression system for coexpression of two different subunits of a recombinant dimeric protein in *E.coli.*

Starting from **D1**, and in view of providing an alternative expression system for expressing the dimeric enzyme nitrile hydratase, the person skilled in the art would be taught to use the two-vector expression system disclosed in either **D2 or D3** with the nitrile hydratase dimeric enzyme and thereby provide the NHase two-vector expression system of the present application.
Thus, the subject-matter of **claims 1-10** does not involve an inventive step.

The catalytic activity of nitrile hydratase is well known in the art (see page 2 of the description, scheme 1). Therefore, the subject-matter of **claim 11** does not involve an inventive step.

SECTION VI

5. The following patent document has been referred to under **Rule 64.3 PCT** and is therefore cited under **Rule 70.10 PCT**:

WO 04/067738, published on 12.04.08, filed on 17.01.04 and having the priority date of 27.01.03.

SECTION VIII

6. The present application does not satisfy the criterion set forth in **Article 6 PCT** because the following claims are not clear.

6.1. **Claim 1** states that "...the expression system comprises in each case at least one plasmid containing at least one nucleic acid sequence encoding the respective subunit".

1 - "At least on plasmid" does not seem to be correct because the present application refers to two-vector expression system, comprising two separate, different vectors, one for each of the NHase subunits alpha and beta. This should be reflected in the wording of claim 1.

2 - When the expression system comprises "...at least one plasmid containing at least one nucleic acid sequence encoding the respective subunit", it includes an expression system comprising one plasmid containing two nucleic acid sequences encoding the respective subunits, i.e. the one-vector NHase expression system well known in the art. In this case the claims lack novelty over **D1**.

3 - The meaning of the expression "in each case" in the context of the wording of claim 1 is not clear.

For the reasons above, **claim 1** lacks clarity.
In case the deficiency in point 2 above is not overcome, an objection of lack of novelty will be pursued.

6.2. In **claim 8** the plasmid designation "PET" is incorrect and should be replaced by "pET".

6.3. The applicant is informed that expressions like "optionally" (**claim 11**) have no limiting effect on the scope of the claims, that is to say, the features following any such expressions are to be regarded as entirely optional (see the **Guidelines for Preliminary Examination (PCT) CIII 4.6**).

********** * **********

7. Final Remarks

In case of filling a request for examination of the present international application, the Applicant is requested to take account of the above comments.

Moreover, in case of filling amended claims, the attention of the Applicant is drawn to the fact that the application may not be amended in such a way that it contains subject-matter which extends beyond the content of the application as filed (**Article 34 (2)(b) PCT** and **Rule 70.2(c) PCT**).
In order to facilitate the examination of the conformity of the amended application with the requirements of **Article 34 (2)(b) PCT**, the Applicant is requested to clearly identify the amendments carried out, irrespective of whether they concern amendments by addition, replacement or deletion, and to indicate the passages of the application as filed on which these amendments are based.